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Glutamate/NMDA Receptor Ion Channel Purification, Molecular Studies & Reconstitution into Stable Matrices.

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12a. DISTRIBUTION / AVAILABILITY STATEMENT

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13. ABSTRACT *(Maximum 200 words)*

This is the final report of a project that had as its primary focus the isolation of the NMDA receptor protein complex and the reconstitution of these proteins for the development of biosensors. The progress made in all phases of this work has met the goals selected for the research. 1) Two proteins of the receptor were fully purified and characterized. 2) The intact complex was purified and biochemically characterized. 3) One of the protein cDNA's was cloned and sequenced. 4) The complex was reconstituted into liposomes and planar lipid bilayers. 5) A prototype sensor based on this receptor complex was developed. 6) Cell model systems for the study of the receptor were developed.

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TITLE

GLUTAMATE/NMDA RECEPTOR ION-CHANNEL PURIFICATION, MOLECULAR STUDIES & RECONSTITUTION INTO STABLE MATRICES

TYPE OF REPORT: FINAL

AUTHORS: ELIAS K. MICHAELIS, PRINCIPAL INVESTIGATOR

DATE: 8/29/91

U.S. ARMY RESEARCH OFFICE

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NAME OF INSTITUTION:University of Kansas

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FINAL REPORT OF SCIENTIFIC WORK

STATEMENT OF THE PROBLEM

The primary goals of this research were to:

1. Isolate and identify glutamate receptor-ion channel protein complexes.

2. Clone the cDNA for these proteins.

3. Develop immunochemical and immunohistochemical techniques for the identification of such receptor proteins and for rapid isolation.

4. Use the techniques developed for reconstitution and ion channel measurements with synaptic membrane proteins and isolated glutamate receptor proteins to determine channel characteristics and membrane stabilization.

5. Develop prototype biosensors based on ion channel amplification characteristics.

6. Use cell models to study the regulation of expression and function of glutamate/NMDA receptors.

SUMMARY OF THE MOST IMPORTANT RESULTS

1. Isolation and Identification of Glutamate Receptor-Ion Channel Complexes.

We first isolated two proteins which appear to be part of the glutamate/NMDA receptor-ion channel complex, a glutamate binding protein of ~70 kDa and a CPP binding protein of ~58 kDa protein (papers published in the Journal of Biological Chemistry). We observed two smaller proteins co-purifying with the glutamate and CPP binding proteins (molecular masses of 42 and 36 kDa). When all four proteins (the 70, 58, 42, and 36 kDa bands) are present in our isolated fractions, we can demonstrate all the expected activities of the NMDA receptor-ion channel complex, i.e., ligand binding for NMDA, glutamate, glycine, PCP, MK801, CPP, and the polyamines (Kumar et al. paper accepted for publication in Nature).

2. Cloning the cDNA for the Proteins.

We have cloned and sequenced a full-length cDNA (1.7 kb) for the largest subunit of the NMDA receptor complex, the glutamate-binding protein. The inferred sequence of the protein describes a unique protein structure with four transmembrane domains. The protein expressed in E. Coli reacts with the antibodies raised against the brain glutamate-binding protein, binds L-glutamate with the same affinity and specific activity as the brain protein, and has been purified by glutamate affinity chromatography (Kumar et al., Nature, in press).

The cDNA for this protein was subsequently used to screen additional cDNA libraries. A series of clones were identified and are being sequenced. In addition, the original clone was inserted into a phage that can be used for stable expression in insect cells through the baculovirus infection process.

3. Development of Immunochemical and Immunohistochemical Techniques for Identification of Receptor Proteins and Rapid Protein Isolation.

A paper was published on the characterization of polyclonal antibody reactivity with glutamate-binding protein sites in rat brain (Eaton et al., 1990). Two other papers are now in press that deal with the development of mouse polyclonal and monoclonal antibodies, their characterization and their use in monitoring the expression of NMDA receptors (Wang et al., 1991; Mattson et al., 1991). These antibodies have become extremely sensitive probes of the appearance and function of NMDA receptors during early development and during degeneration.

4. Reconstitution and Ion Channel Measurement with Purified Proteins to Determine Channel Characteristics and Membrane Requirements.

We successfully achieved reconstitution of the 4 subunits of the NMDA receptor and demonstrated with rapid kinetics instrumentation that they exhibit glutamate-stimulated ion fluxes in liposomes. The pharmacological properties of this channel activity are most consistent with those of the NMDA receptor subtype. Furthermore, the Ab's raised against the 70 kDa glutamate-binding protein dramatically alter the kinetics of the ion flux response to NMDA (Ly and Michaelis, 1991). Reconstitution of this protein complex in planar lipid bilayers and direct measurement of glutamate-activated currents were also accomplished (Uto et al., 1990). The results are providing further confirmation of the involvement of the four protein subunits in the formation of an active NMDA receptor-ion channel complex (Aistrup et al., in preparation).

5. Development of Prototype Receptor-Based Biosensors

The success of reconstitution of the NMDA receptor proteins into planar lipid bilayers led to the use of this system in the detection of glutamate levels as low as 10^{-8} M. These studies were summarized in a recent article accepted for publication (Minami et al., Analytical Chemistry) and they include a characterization of the sensitivity, stability and selectivity of such a sensor system.

6. Cell Models for the Study of NMDA Receptor.

Primary neuronal cultures and sophisticated fluorescence ratio imaging techniques were developed to monitor the activity of NMDA receptors. These methods are used to explore different chemical modification procedures on receptor structure and function. These studies will be continued with cloned and transiently expressed receptors in different mammalian and insect cell lines.

PUBLICATIONS THAT HAVE RESULTED FROM THIS WORK

E. K. Michaelis, J.-W. Chen, T. M. Stormann, S. Roy and E. LeCluyse. Molecular and immunochemical properties of a putative glutamate receptor protein. In Neurotransmitters and Cortical Function, 1988, M. Avoli, Ed., Plenum Press, New York.

E. K. Michaelis, V. Thai, S. Gosh, S.L. Early and Decedue, C. Purification of an inhibitor of glutamate binding sites in synaptic membranes from the venom of Araneus gemma. Invited Chapter for Neurotoxicology, 1988, G. G. Lunt, Ed., pp. 83-89.

M. Uto, E. K. Michaelis, I. F. Hu, Y. Umezawa and T. Kuwana, Biosensor development with a glutamate receptor-ion channel reconstituted in a lipid bilayer. Analytical Sciences, 1990, 6, 221-225.

M. L. Michaelis and E. K. Michaelis. Functional parameters of ATPases from brain subcellular membranes, in Advances in Membrane Fluidity, vol. 4 : Information, Storage and Membrane Transport (Eds. R. C. Aloia, C. C. Curtain, and L. M. Gordon), 1990, Alan R. Liss, New York, pp. 133-163.

M. J. Eaton, J.-W. Chen, K. N. Kumar, Y. Cong and E. K. Michaelis, Immunochemical characterization of brain synaptic membrane glutamate-binding proteins. J. Biol. Chem., 1990, 265, 16195-16204.

G. Hoel, M. L. Michaelis, W. J. Freed and J. E. Kleinman. Characterization of Na^+-Ca^{2+} exchange activity in membrane vesicles from post mortem human brain. Neurochem. Res., 1990, 15, 881-887.

M. D. Cunningham and E. K. Michaelis. Solubilization and partial purification of 3((±)-2-carboxypiperazine-4-yl)-[1,2^{3}H]propryl-1-phosphonic acid ([^{3}H]CPP) recognition proteins from rat brain synaptic membranes. J. Biol. Chem., 1990, 265, 7768-7778.

K. N. Kumar, K. T. Eggeman, J. L. Adams and E. K. Michaelis. Hydordynamic properties of the purified glutamate-binding protein subunit of the N-methyl-D-aspartate receptor. J. Biol. Chem., 1991, 266, 14947-14952.

A. M. Ly and E. K. Michaelis. Solubilization, partial purification and reconstitution of glutamate and N-methyl-D-aspartate-activated cation channels from brain synaptic membranes. Biochemistry, 1991, 30, 4307-4316.

E. K. Michaelis and M. L. Michaelis. Molecular aspects of glutamate receptors and sodium-calcium exchange carriers in mammalian brain: Implications for neuronal development and degeneration. Neurochem. Res., 1992, 17, 29-34.

Eight papers that have resulted from this work are currently "in press" awaiting final publication.

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REPORT OF INVENTIONS

None